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Bradykinin receptors in the guinea-pig taenia caeci are similar to proposed BK_3 receptors in the guinea-pig trachea, and are blocked by HOE 140

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1 Bradykinin (BK) receptors of the guinea-pig taenia caeci were compared with those of the guinea-pig trachea, a preparation proposed to possess novel BK_3 receptors.

2 Bradykinin-evoked contractile responses were unaffected in both preparations by the selective BK_1 receptor antagonist [des-Arg^9,Leu^8]-BK (1 μM–10 μM). The BK_2 receptor antagonists, D-Arg-[Hyp^3,D-Phe^7]-BK and D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-BK, both had low affinities (apparent pK_B estimates < 6) which did not differ significantly between the two preparations ($P > 0.05$). In contrast, the novel bradykinin receptor antagonist D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK (HOE 140) potently antagonized responses to bradykinin with relatively high affinity (apparent $pK_B = 8.42 \pm 0.15$ and 8.94 ± 0.16 in the taenia caeci, and trachea, respectively).

3 We conclude that the bradykinin receptors in the guinea-pig taenia caeci have similar recognition properties to those present in the guinea-pig trachea, and in this respect the taenia caeci represents a useful preparation for the further study of proposed novel BK_3 receptors.

Keywords: Bradykinin; BK_3-receptors; trachea (guinea-pig); taenia caeci (guinea-pig); HOE 140; D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK; bradykinin antagonist; bradykinin receptors

Introduction

Bradykinin (BK) receptors were originally divided into BK_1 and BK_2 subtypes (see Regoli & Barabé, 1980). Recently, Farmer and colleagues reported that both the selective BK_1 receptor antagonist [des-Arg^9,Leu^8]-BK (Regoli & Barabé, 1980), and also certain [D-Phe^7]-BK substituted analogues (Vavrek & Stewart, 1985) are rather inactive against BK-evoked contraction of the epithelium-denuded guinea-pig trachea, and did not displace [^{3}H]-BK binding in tracheal smooth muscle membrane preparations (Farmer *et al.*, 1989). These observations led to the proposal by this group, of a novel BK receptor subtype which they termed BK_3. To date, the guinea-pig trachea is still the only convenient *in vitro* assay preparation for the study of proposed BK_3 receptors. However, this preparation suffers from a number of disadvantages in that responses vary considerably depending on the involvement of epithelium-dependent factors and prostaglandins (Bramley *et al.*, 1990), contractions are slow and small in tension, and this preparation is rather insensitive to BK so that determination of the maximum response is difficult.

Our preliminary studies (Field *et al.*, 1988) have shown that a number of [D-Phe^7]-BK substituted antagonist analogues, as well as [des-Arg^9,Leu^8]-BK, have low affinity also in the guinea-pig taenia caeci as compared to a number of other preparations, thereby suggesting similarities between the bradykinin receptors of the guinea-pig taenia caeci with those proposed as 'BK_3' receptors in the guinea-pig trachea. In contrast to the guinea-pig trachea, contractile responses to bradykinin in the guinea-pig taenia caeci have the advantage that they appear to be largely due to a direct action on these strips of pure longitudinal smooth muscle and responses are large in tension, with multiple replications possible within individual preparations.

The aim of this study, therefore, was to compare the recognition properties of the BK receptors mediating contraction of the guinea-pig taenia caeci, with the proposed BK_3 receptors in the guinea-pig trachea, by functional studies in isolated preparations. The effect of BK receptor antagonists, including the novel analogue D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK (HOE 140), the most potent of a series recently described (Hock *et al.*, 1991; Lembeck *et al.*, 1991), were used to compare receptor recognition characteristics.

A preliminary account of these results has been communicated to the British Pharmacological Society (Hall *et al.*, 1991).

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Methods

General

Male Dunkin Hartley guinea-pigs (450–800 g) were killed by cervical dislocation, and the taenia caeci and trachea removed and cleared of superficial blood vessels and connective tissue. The trachea was opened longitudinally, the epithelium was removed by gentle rubbing with a cotton wool bud (see, Goldie *et al.*, 1986) and three rings were separated in the cartilage ring to yield preparations for isometric recording in the radial direction. An absence of relaxant responses to carbachol and BK was taken as indicating that the preparations had been functionally denuded of epithelium (Goldie *et al.*, 1986; Farmer *et al.*, 1987; Bramley *et al.*, 1990). Of the three possible taenia strips, the two without mesenteric attachment were separated from underlying circular muscle with fine scissors, and mounted longitudinally. Both preparations were mounted on a micrometer-controlled isometric assembly that allowed precise length adjustments. An initial resting tension of 500 mg was applied, and tissues allowed to develop spontaneous tone. In the taenia caeci, preparations that developed additional tone ('high tone preparations') to BK tended to produce a biphasic response, whereas in those that did not, contractile responses only were observed. Tension was measured by means of Grass FT03B force-displacement transducers coupled to JJ Instruments Cr342 potentiometric flat-bed recorders.

Experiments were carried out in Krebs solution (composition mM: Na^+ 140, K^+ 5.9, Cl^- 104.8, $H_2PO_4^-$ 1.2, HCO_3^- 24.9, Ca^{2+} 2.6, Mg^{2+} 1.15, SO_4^- 1.15, glucose 10), maintained at 37°C and oxygenated with 95%O_2:5%CO_2. The Krebs solution contained atropine, mepyramine, cimetidine, guanethidine, (all 1 μM) and hexamethonium (10 μM).

Antagonist pK_B estimates

In preparations of taenia caeci or trachea, concentration-response curves for BK were obtained using non-cumulative doses in a randomised block design, with responses obtained in the presence of BK receptor antagonist (test) or no antagonist (concurrent control). A 12 min BK dose-cycle was used in conjunction with a 10 min antagonist pre-test incubation time. Other experiments were carried out to test for any effect on pK_B estimates for D-Arg-[Hyp^3,D-Phe^7]-BK of peptidase inhibitors. The kininase I inhibitor DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid (MERGETPA), the kininase II inhibitor enalaprilat and the neutral endopeptidase EC 3.4.24.11 inhibitor phosphoramidon (all 1 μM) were used. A similar protocol to that described above was used, but with matched preparations bathed in Krebs solution containing the peptidase inhibitors (present from the start of tissue equilibration), or control.

Specificity of antagonism by D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK

The effect of D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK (1 or 10 μM) was tested against responses to submaximal concentrations of substance P, neurokinin A, angiotensin II and carbachol. Control responses were obtained and then repeated following a 10 min incubation period with antagonist.

Source of drugs

Agents were obtained as follows: carbamylcholine chloride, atropine sulphate and hexamethonium bromide (Sigma, UK), mepyramine maleate (May and Baker, U.K.), cimetidine (Smith, Kline and French, U.K.), guanethidine sulphate (Ciba, U.K.), enalaprilat (Merck, Sharp & Dohme, New Jersey, U.S.A.), phosphoramidon, angiotensin, neurokinin A, substance P (Peninsula) bradykinin, D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-BK, D-Arg-[Hyp^3,D-Phe^7]-BK, (Bachem, U.K.). D-Arg-[Hyp^3, Thi^5,D-Tic^7,Oic^8]-BK (HOE 140) was a kind gift from Dr A. Hallett, Sandoz Institute for Medical Research, London. All salts used were of analytical grade and were obtained from B.D.H.

All agents were dissolved in distilled water and peptides were stored deep frozen under N_2.

Expression of results and statistical analysis

Contractile responses were normalised in each preparation in terms of maximal carbachol contractions, and the estimates are shown as means ± s.e.mean. Tests for significance were made with Student's t test for two independent samples. The apparent pK_B estimates and their s.e.mean were obtained from individual dose-ratio estimates (x) between test and control preparations, by calculation from the Gaddum-Schild equation, $pK_B = \log_{10}(x - 1) - \log_{10}[A]$, where [A] is the antagonist concentration. In the guinea-pig trachea, lateral shifts of the lower half of the log concentration-response curve only were used for calculation of apparent pK_B values (see Results).

Results

Isolated tissue studies

Both preparations contracted in response to BK. 'High tone' taenia caeci preparations showed an initial relaxant response, but this was never seen in the tracheal preparations (see Figure 1). In both preparations, the threshold concentration of BK causing contraction was *ca.* 0.3 nM. The log concentration-response curve to BK for contraction of the taenia caeci appeared in individual preparations to be monophasic, with a clearly defined maximal response obtained at concentrations 10 μM or lower. In contrast, in the trachea, two phases of contraction were evident on individual and meaned log concentration-response curves for BK (see control curve of Figures 2 and 3). With regard to the main purpose of the present study, the second phase begins above the highest concentration used by Farmer *et al.* (1989) (1 μM) in their analysis of the 'BK_3' receptor. For present purposes, therefore, apparent pK_B estimates for the antagonists in the tracheal preparation were determined only for the lower portion of the curve that corresponds to that portion of the BK response curve studied by Farmer's group, rather than the upper portion of the curve where greater shifts with D-Arg-[Hyp^3, Thi^5,D-Tic^7,Oic^8]-BK were seen.

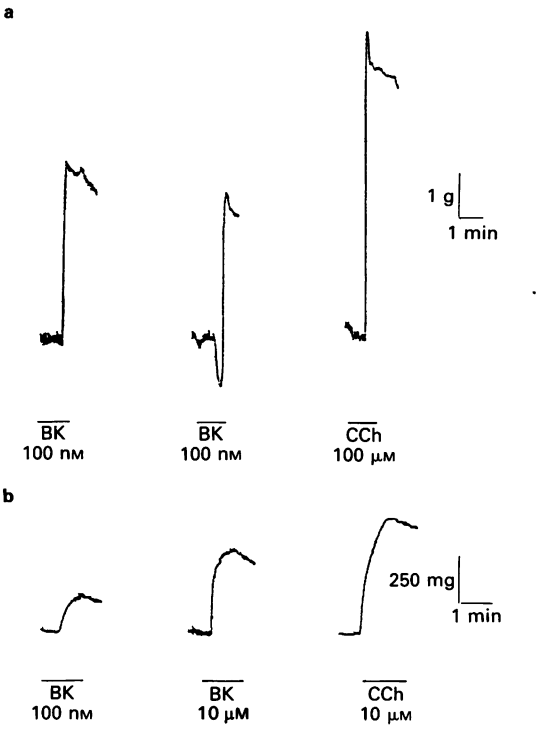

Figure 1 Typical traces of isometric tension recordings in (a) the guinea-pig taenia caeci, and (b) the epithelium-denuded trachea, in response to bradykinin (BK) and carbachol (CCh). Note the initial relaxant response which is sometimes seen in high-tone taenia caeci preparations. Bars indicate the periods of drug application.

None of the BK_2/BK_3 antagonists had partial agonist activity. The selective BK_1 receptor agonist [des-Arg^9]-BK was inactive (data not shown) and the BK_1 receptor antagonist [des-Arg^9,Leu^8]-BK (1–10 μM) did not antagonize responses to BK in either the guinea-pig trachea or taenia caeci (Table 1) and showed partial agonist activity at higher concentrations ($\geq$ 10 μM). The BK analogues D-Arg-[Hyp^3,D-Phe^7]-BK and D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-BK, though having no appreciable activity at 3 μM, at 10 and 30 μM produced shifts to the right of the BK log concentration-response curves in both preparations (Figure 2). The apparent pK_B values estimated from these shifts are shown in Table 1. For all antagonists the range of concentrations that could be used was not wide enough to warrant full Schild plot analysis, but since calculated individual pK_B estimates did not differ significantly ($P > 0.05$) with antagonist concentration, these values were pooled, and are described in the text and Table 1 as apparent pK_B estimates. The apparent pK_B estimates for D-Arg-[Hyp^3, D-Phe^7]-BK were not significantly different when estimated in the presence of the carboxypeptidase inhibitor MERGETPA, the kininase II inhibitor enalaprilat and the neutral endopeptidase inhibitor phosphoramidon (each 1 μM: $n = 4$; $P > 0.05$, data not shown).

D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK (30 nM–300 nM) potently antagonized contractile responses to BK in the guinea-pig taenia caeci and trachea (see Figure 3). At 300 nM D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK, depression of the maximal

Table 1 Affiinity estimates of bradykinin (BK) receptor antagonists in the guinea-pig trachea and taenia caeci

	Apparent pK_B		
Antagonist	*Taenia caeci* (± s.e.mean; *n*)	*Trachea* (± s.e.mean; *n*)	*Difference* (± s.e.mean)
[des-Arg^9,Leu^8]-BK	<5.0 (9)	<5.0 (9)	
D-Arg-[Hyp^3,D-Phe^7]-BK	5.89 (0.32; 11)	5.94 (0.31; 10)	0.05† (0.45)
D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-BK	5.81 (0.35; 8)	5.87 (0.22; 7)	0.06† (0.36)
D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK	8.42 (0.15; 12)	8.94 (0.16; 14)	0.52* (0.21)

Apparent affinities were calculated from individual dose-ratios (x) using the Gaddum-Schild equation $pK_B = \log_{10}(x - 1) - \log_{10}[A]$, where [A] is the antagonist concentration. Abbreviations: n = number of estimates; <5.0 denotes inactive at 10 μM.
† $P > 0.05$; * $P < 0.05$.

response obtained to BK was evident, so the apparent pK_B values shown in Table 1 were estimated only from individual dose-ratios obtained at 30 nM and 100 nM antagonist. The initial relaxation seen with BK in high-tone taenia caeci preparations was found to be antagonized to an equivalent extent (data not shown).

D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK (1 μM) appeared selective in its action since it was inactive against responses to submaximal concentrations of substance P, neurokinin A, carbachol and angiotensin II in both preparations ($n = 4$; data not shown).

Discussion

Results from this study show that BK receptors mediating contraction of the guinea-pig taenia caeci have recognition properties very similar to those mediating contraction of the guinea-pig trachea. In the present study, log concentration-response curves to BK in the taenia caeci were monophasic, whereas those in the guinea-pig trachea were evidently biphasic. Whether in the trachea these phases represent the existence of two receptor subtypes, or coupling mechanisms, within this preparation will be the subject of further investigation.

The bradykinin receptors in these two preparations are certainly not of the BK_1 subtype in view of the lack of activity of the BK_1-selective ligands [des-Arg^9]-BK and [des-Arg^9,Leu^8]-BK. Furthermore, antagonists of the [D-Phe^7]-BK series had low affinity ($pK_B < 6$) as compared to that measured in a wide variety of preparations regarded as expressing BK_2 receptors ($pK_B > 7.0$) (Griesbacher *et al.*, 1987; Field *et al.*, 1988; Hall & Morton, 1991a). The low pK_B values obtained with the [D-

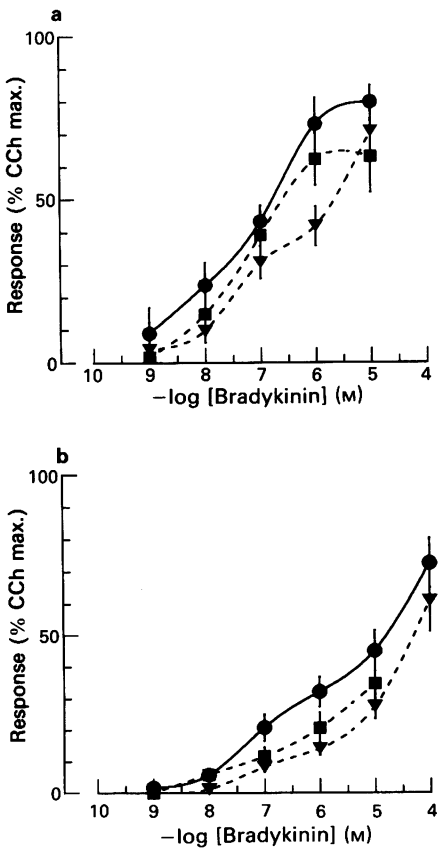

Figure 2 Antagonism by D-Arg-[Hyp^3,D-Phe^7]-BK of the contractile responses to bradykinin in (a) guinea-pig taenia caeci and (b) the guinea-pig trachea. Curves shown are control (●), or in the presence of antagonist at 3 μM (■) and 10 μM (▼). Each point is the mean taken from 9–12 preparations; s.e.mean shown by vertical lines.

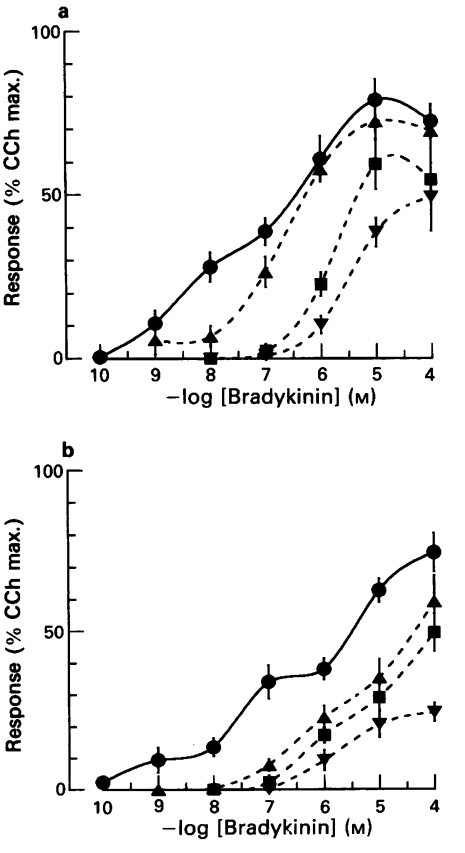

Figure 3 Antagonism by D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK of the contractile responses to bradykinin in (a) guinea-pig taenia caeci and (b) the guinea-pig trachea. Curves shown are control (●), or in the presence of antagonist at 30 nM (▲), 100 nM (■) and 300 nM (▼). Each point is the mean taken from 9–12 preparations; s.e.mean shown by vertical lines.

Phe^7]-BK substituted analogues are unlikely to be due to degradation by peptidases since the pK_B for D-Arg-[Hyp^3,D-Phe^7]- BK was unaffected by the presence of peptidase inhibitors.

In contrast to the [D-Phe^7]-BK substituted analogues, potent antagonism of responses to BK by D-Arg-[Hyp^3,Thi^5, D-Tic^7,Oic^8]-BK was seen in the trachea and taenia caeci (apparent $pK_B = 8.9$ and 8.4 respectively). This finding with D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK in the guinea-pig trachea confirms a preliminary report by Perkins *et al.* (1991). Though these apparent pK_B estimates for D-Arg-[Hyp^3,Thi^5,D-Tic^7, Oic^8]-BK differed statistically ($P < 0.05$), it should be pointed out that strict competition at equilibrium was not established with this compound in the present experiments, and further, it is evident that a second receptor site (or mechanism) is present in the trachea. In pharmacological terms, therefore, we do not consider the difference in apparent pK_Bs as being particularly significant. It is also interesting to note that the pK_B values are approximately one log unit lower than the pA_2 value for D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK described in typical BK_2 preparations, the rat uterus ($pA_2 = 9.74$; Perkins *et al.*, 1991), and the rat duodenum ($pA_2 = 10.0$; unpublished data) and the rabbit iris spincter pupillae ($pA_2 = 10.5$; Everett *et al.*, 1991).

Given that [D-Phe^7]-BK antagonists and D-Arg-[Hyp^3, Thi^5,D-Tic^7,Oic^8]-BK have lower affinities in the taenia and trachea as compared with typical BK_2 preparations, these results would support the proposal by Farmer *et al.* (1989) that BK receptors with properties differing from typical BK_2 receptors do exist. It may be noted however, that similar pK_B values for D-Arg-[Hyp^3,D-Phe^7]-BK, D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-BK and D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK have been reported in further preparations taken from the guinea-pig: the ileum (Birch *et al.*, 1991; Perkins *et al.*, 1991), and the urinary bladder (Maggi *et al.*, 1989). Thus both series of antagonists give affinity data suggesting a common difference between BK receptor of the guinea-pig preparations as compared to preparations from other species. These observations highlight the possibility that differences in antagonist affinities may reflect species-related differences in bradykinin receptor subtypes.

In conclusion, the predominant receptor type in the guinea-pig taenia caeci is very similar to the novel receptor type in the guinea-pig trachea which has been described as a BK_3 subtype (Farmer *et al.*, 1989). Whether the properties of these receptors justify the creation of a distinct receptor class should, however, perhaps depend on evidence from other studies, such as cloning, coupling studies or the development of BK_3 selective antagonists. It is of great interest that these novel receptors, both in guinea-pig airways and gastrointestinal smooth muscle, are effectively blocked by D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK a potent example of a recently developed analogue series (see also Farmer *et al.*, 1991). For further study of the 'BK_3' receptor, the taenia would seem to have a number of advantages over the tracheal preparation. There appears to be only one receptor type, there are good responses in terms of tension developed and replication of responses within individual preparations. Further since the taenia consists of pure smooth muscle it is very suitable for mechanistic studies (Hall & Morton, 1991b).

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